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(54) Title: PURIFIED RESISTANT STARCH PRODUCTS AND THEIR PREPARATION

(57) Abstract

Processes and products containing purified resistant starch with high resistant starch content. The processes include forming a water-starch suspension. The water-starch suspension is heated to temperatures above 100°C and cooled to form a crude starch gel. The gel is comminuted and mixed with an amylase. The amylase digests non-resistant starch fractions leaving resistant starch. The amylase is inactivated, such as by heat treatment above 100°C. Resistant starch is concentrated by washing and separating the non-solubilized resistant starch fraction, such as by centrifugation. Purification, such as with water and/or ethanol improves flavor, odor, and taste. The purified resistant starch products can be used as additives in novel food products to substitute for conventional dietary fiber sources, reduce caloric content, bind water, and absorb heat. Concentrated resistant starch can also be administered orally as powders, tablets, capsules, or caplets as a dietary supplement affecting gastrointestinal tract function.

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DESCRIPTION

PURIFIED RESISTANT STARCH PRODUCTS AND THEIR PREPARATION

Technical Field

The technical field of this invention includes processes for producing purified resistant starch, purified resistant starch products, and dietary foods and substances containing the purified resistant starch products.

Background Art

Starches from cereal grains, legumes, potatoes and other sources undergo a starch conversion when heated and subsequently cooled. This conversion process, known as starch retrogradation, includes the formation of resistant starch (RS) which resists enzymatic breakdown in vitro and in the small intestine of man. Resistant starch is in general not present in uncooked starch-containing foods but is formed as a result of food processing. The amount of resistant starch in cooked or baked foods such as bread, pasta, cereals, legumes, and potatoes is small; it appears in concentrations below 3%.

The current theory of resistant starch formation views the process as retrogradation or recrystallization of the amylose fraction of starch. Gelatinization, i.e. dissociation of starch granules by heat-moisture treatment, constitutes a prerequisite to generation of resistant starch. Upon cooling of gelatinized starch, dispersed amylose molecules reassociate spontaneously. The resulting amylose crystallites so formed are resistant to digestion by amylolytic enzymes but can be solubilized using potassium hydroxide or dimethylsulfoxide (DMSO).

Physiological studies have shown similarities between RS and certain types of dietary fiber with respect to the manner in which these materials are processed in the human gastrointestinal tract. RS is undigestible in the small intestine but is acted upon by microorganisms present in the large intestine to produce by-products, such as short-chain fatty acids, which are of significance in improving colon function. A diet high in resistant starch was shown to protect significantly against diverticulosis. Indications exist according to which undigested starch constitutes an important factor in protection against colon carcinogenesis.

In the opinion of some researchers, resistant starch combines the physiological benefits of insoluble and soluble dietary fiber. In addition to its dietary fiber effect, RS also appears to be potentially valuable as a means for reducing the utilizable caloric content of foods. RS is not hydrolyzed and absorbed in the upper intestinal tract. Thus it can be used to produce low calorie food products while potentially having desirable benefits in the digestive tract. To date however, this has not been feasible because of the relatively low

concentrations of resistant starch and the unsatisfactory nature of the prior art isolation processes.

The typical prior art process for increasing the concentration of resistant starch employed numerous heating and cooling steps of a starch gel which result in increasing amounts of RS. However, such repeated heating and cooling treatments have derogatory effects on the flavor, color, taste, and other quality characteristics of the end product. Such techniques are costly, time consuming, and the resulting products have never exceeded 50% resistant starch.

Another approach to RS preparation involves the use of amylolytic enzymes to remove readily degradable starch structures leaving intact RS. The inventors of the present invention have discovered that some processes for resistant starch production which include the use of enzymes suffer substantial problems because the enzymes remain in the product in significant amounts leading to undesirable odor formation when stored and unacceptable problems due to continued enzyme activity when food products (including baked ones) contain such resistant starch. Thus there remains a need in the art for improved processes for the production of resistant starch.

The present invention presents novel processes for the preparation of purified resistant starch products with high amounts of resistant starch. Resistant starch products can be successfully used in the production of dietary fiber enriched and/or calorie reduced food stuffs and as dietary supplements. The novel process provides resistant starch products which can be stored over long periods at ambient temperatures, and do not have offensive color, flavor, and/or other taste components objectionable for use in food products in relatively large amounts as a food additive.

Disclosure of Invention

This section is a summary disclosure of the invention and is supplemented by the description of the best modes give hereinafter. The invention includes novel purified resistant starch products having at least 50 percent resistant starch content produced from a variety of starch materials, preferably materials which are relatively high in amylose content. The high concentration resistant starch products are advantageously produced by novel processes of this invention which preferably include mixing starch-containing material and a solvent, such as water, to form a mixture, more preferably a suspension. The starch and water or other mixture or suspension is heated and then cooled for sufficient time to cause starch retrogradation and formation of resistant starch fractions.

The resulting crude starch gel is comminuted and/or mixed, or otherwise treated with amylase or other suitable amylolytic enzyme to cause hydrolysis or other digestion of

non-resistant starch fractions. The amylolytic enzyme is preferably inactivated in a suitable manner, such as by heat treatment. The non-resistant starch fractions digested by the enzyme or other suitable treatment are preferably solubilized by washing with a suitable solvent, such as water, water and ethanol, or ethanol. Such solubilizing agent or agents and solubilized fractions can be removed by suitable treatment, such as centrifugation to concentrate the resistant starch fractions. Additional purification can be effected by particle density gradation and other suitable grading techniques performed one or more times. The product can be dried, preferably at relatively low drying temperatures to prevent degradation.

The novel purified resistant starch products are useful in a variety of novel food products, for food processing purposes, dietary supplementation purposes, and for effecting gastrointestinal function, as described elsewhere herein.

Brief Description of the Drawings

Fig. 1 is an electron micrograph showing raw amylomaize VII starch granules.

Fig. 2 is an electron micrograph showing amylomaize VII starch after 1 autoclaving/cooling cycle.

Fig. 3 is a graph showing melting enthalpy of the starch pictured in Fig. 2

Fig. 4 is an electron micrograph showing amylomaize VII starch after 4 autoclaving/cooling cycles.

Fig. 5 is a graph showing melting enthalpy of the starch pictured in Fig. 4.

Fig. 6 is an electron micrograph showing particles of a vacuum-dried purified resistant starch product produced after 4 autoclaving/cooling cycles in accordance with this invention.

Fig. 7 is a graph showing melting enthalpy of the starch product pictured in Fig. 6.

Fig. 8 is an electron micrograph showing particles of resistant starch removed in a process in accordance with this invention.

Fig. 9 is a graph showing melting enthalpy of the product pictured in Fig. 8.

Fig. 10A is a series of graphs showing melting enthalpies of freeze-dried amylomaize VII starch subjected to different heat-moisture treatments.

Fig. 10B is a series of graphs showing melting enthalpies of purified resistant starch products produced in accordance with the invention. The purified resistant starch products were produced from the four different heat-moisture treated starches as graphed in Fig. 10A.

Fig. 11 is a graph showing the effects of variant production processes on the resistant starch content of the preparations.

Best Modes for Carrying Out the Invention

The invention includes novel processes for the production of purified resistant starch. The processes use an appropriate source of starch-containing material from which the

resistant starch can be formed. Preferred sources selected for use are starches high in amylose content, greater than 50%, more preferably greater than 70%. Suitable starches include amylomaize VII starch, high amylose pea starch, and high amylose barley starch. Alternative types of starch suitable for use in the novel processes include starches from other vegetable sources, including cereals, legumes, and tubers. Still other forms of vegetable starches may also be appropriate for use in the invention. Commercially available starches are suitable.

The starch or mixture of starches used in the processes of this invention are mixed and preferably suspended in water in a suitable ratio to prepare a water-starch suspension. The ratio of starch to water is approximately 1:2 to 1:20, more preferably 1:3 to 1:10, starch:water. Relatively higher resistant starch formation is achieved with ratios having greater proportion of starch. However, the gelatinization process requires excess water and a diminishing effect can occur due to this factor as starch content is increased. The formation of the starch-water suspension can be accomplished using water at ambient temperatures, for example 10°-50°C.

The water-starch suspension is heated, preferably in an autoclave at temperatures above 100°C to ensure full starch gelatinization. The heating is preferably carried out at temperatures in the range 110-160°C, more preferably 120-135°C. The heating is advantageously done at elevated pressures, such as in an autoclave at 10-15 psi gauge, or the saturated steam pressure associated with the heating process at such temperatures.

The amount of time needed to heat process the starch-water mixture will necessarily vary due to heat transfer considerations associated with differing sizes of batches, etc. Heating periods of 30 minutes to 2 hours are believed appropriate, wherein the mixture has reached the indicated autoclave temperatures. After the starch-water mixture has been suitably heat processed as indicated above, the mixture is cooled to allow amylose retrogradation to take place. Storage at 4°C for periods of approximately 2 hours or greater will typically cause starch retrogradation. Overnight storage at this temperature is sufficient in most cases.

If desired, the starch gel formed can be stored for substantial periods of time at temperatures of 4°C or colder. Such storage does not appear to have significant impact on the resistant starch formation and stability.

Table 1 shows results for a number of different starch materials which can be used to form resistant starch, and the approximate concentrations of the resistant starch in the crude heat-moisture treated starch preparations processed such as described above. Comparative information showing the resistant starch content of corresponding RS products

from a number of these different raw starch materials is also shown below in Table 5, as described in Example 11.

TABLE 1

YIELDS OF RESISTANT STARCH (RS)

Starch		RS(%)
Amylomaize VII	(70% amylose)	21.3
Amylomaize V	(53% amylose)	17.8
Pea starch	(33% amylose)	10.5
Wheat starch	(25% amylose)	7.8
Maize starch	(26% amylose)	7.0
Potato starch	(20% amylose)	4.4
Waxy maize	(<1% amylose)	2.5

Starch:water ratio 1:3.5 for amylomaize VII and 1:2.0 for others.

Autoclaving Temperature: 134°C.

Autoclaving Time: 1 hour.

The inventors of the present invention developed novel processes which include the use of highly effective amylolytic enzymes to isolate resistant starch formed during retrogradation. One of the distinct advantages of the current invention is the elimination of the need for repeated autoclaving and cooling to prepare products with substantial amounts of resistant starch. However, it has been found that a combination of repeated autoclaving/cooling cycles which cause increasing amounts of resistant starch in the crude starch preparation, also increase the effectiveness of the novel methods of this invention and create even higher yields of RS in the end product.

To prepare the starch gel for the subsequent enzymatic treatment it is advantageously comminuted or otherwise broken to divided into particles. This is preferably accomplished using a mechanical device for shearing, shredding, or otherwise breaking the gelatinous masses into small pieces or particles, advantageously under the addition of water. Such

comminution of the starch gel can be accomplished using a wet milling process, blending, or an equivalent.

The prepared starch gel is mixed with a suitable amylolytic enzyme, preferably alpha-amylase (E.C. Number 3.2.1.1). This step is designed to break down the starch not converted to resistant starch during retrogradation. Applications of alpha-amylases from bacterial, fungal, and pancreatic sources were investigated. The most preferred enzyme in terms of effectiveness (high amounts of RS in the end-product) is bacterial alpha-amylase, from Bacillus licheniformis. It outperforms all the other alpha-amylases (Example 3). This enzyme is commercially available in liquid form under the trademarks Termamyl 120-L, Novo Laboratories, Danbury Connecticut; and Takalite, Miles Laboratories, Elkhart, Indiana. This enzyme is included in amounts sufficient to cause degradation of the starch fractions which are not resistant starch, within a desired processing time, and as limited by other considerations. Preferred concentrations of bacterial alpha-amylase are approximately in the range 2-10 milliliters of such commercial enzyme preparations per 100 grams of native starch employed. More preferably, approximately 5 milliliters of enzyme solution per 100 grams of starch are included. Higher amounts produce diminishing increases of resistant starch but result in significant darkening of the end product. Lower concentrations reduce the yield of resistant starch.

The enzyme digestion step is preferably performed at temperatures in the approximate range of 70-100°C. The enzyme digestion process is advantageously carried on for period of approximately 15 minutes to 2 hours, more preferably 30-60 minutes.

It is alternatively possible to use less preferred fungal, cereal, pancreatic, or other alpha-amylases. A tested fungal amylase is sold under the trademark Clarase. The fungal and pancreatic alpha-amylases require different conditions from those described above. They are easier to handle with regard to enzyme inactivation and purification of the end product but are substantially less effective than the bacterial alpha-amylase with regard to yield and quality of RS (Example 3).

The amylase is preferably mixed into the starch-water mixture after comminution. The wet milling will form a homogenous starch-water mixture more susceptible to enzymatic attack. The mixture containing comminuted starch, water, and the enzyme is preferably stirred during the enzymatic digestion process to maintain homogeneity and maximum enzyme activity.

The novel processes according to this invention further include processing to inactivate the amylase enzyme. The preferred method for inactivation is by heat inactivation treatment. The preferred bacterial enzyme indicated hereinabove is rendered inactive by

heat inactivation at temperatures above 100°C. The heat inactivation is preferably accomplished at temperatures in the approximate range of 100°-150°C, more preferably 120°-130°C. The heat inactivation is preferably carried out under autoclave or equivalent conditions. Pressure conditions of approximately 10-15 psi gauge, or saturated steam pressures associated with such autoclaving are thus appropriate in processes according to this invention.

The inactivation, such as by heat processing, should be performed for periods of time sufficient to cause inactivation of the enzyme. In the case of the preferred bacterial alpha-amylase heat inactivation, periods approximately in the range 5-60 minutes are preferred, dependent upon the inactivation temperature used. Insufficient inactivation time or temperature results in residual enzyme activity which can be particularly problematic in the production of baked goods, but also not satisfactory in other contexts as well. Relatively longer heat inactivation times and relatively higher inactivation temperatures cause destruction of the resistant starch and lower process yields.

In the novel methods of this invention concentration and purification are performed on the enzyme digested starch mixture to selectively segregate the fraction of the mixture which is suitable for use as a purified resistant starch product. As used herein, concentration refers to the removal of starch degradation products formed during enzymatic hydrolysis to thus isolate and concentrate resistant starch. Purification refers to the removal of components such as denatured enzymes which impart an off-flavor to the end product. The amount of RS in the end product depends on the amount of RS in the crude heat-moisture treated starch preparation, consequently, an appropriate source is required; the degree of hydrolysis of non-resistant starch, consequently, an appropriate highly effective enzyme is required; and the removal of digested starch fractions formed during hydrolysis of starch, consequently, appropriate separation steps (washing solutions, centrifugation) are required.

Purification does not significantly affect the amount of RS in the end product and is only designed to remove off-flavor components. Purification might be redundant in case of fungal or pancreatic alpha-amylase. Concentration is required in each case. Concentration and purification can partly be combined.

The preferred separation procedures allow separation of desired portions of the digested water-starch mixture from fractions which are rich in undesired enzyme residue. Such separation can advantageously be initiated by centrifuging the digested water-starch mixture to segregate a starch mass from a watery supernatant, and removing the supernatant. The centrifugation can advantageously be performed using centrifugal accelerations of 1000 g or higher, for period of approximately 10-15 minutes or longer. The resulting concentrated

starch intermediate product is then mixed with a washing solution. The washing solution can be cold water (0°-15°C), hot water (70°-100°C), cold water and ethanol, hot water and ethanol, and other appropriate washing solutions which are not derogatory to the concentration of resistant starch contained in the intermediate product. Washing solutions which contain ethanol produce a relatively whiter product with no significant smell. Washing solutions containing hot water are most effective to remove digested non-resistant starch and thus provide products high in RS. The centrifugation process is then advantageously repeated to again develop a more concentrated and purified resistant starch mass and supernatant. The supernatant is removed and eliminated to cleanse the intermediate starch product from the digested non-resistant starch, enzyme, and other by-products of the enzyme digestion process.

The washing procedures indicated above can be repeated any number of times using a single washing solution, various washing solutions for progressive washing cycles, or continuous washing. For example, the washing can begin with cold water washing cycles followed by warm or hot water washing cycles. Alternatively the pre-inactivation washings can be with washing solutions of water and ethanol in various proportions of such ingredients.

The washing procedures of this invention advantageously include thorough mixing of the centrifuged mass of starch material with the washing solution or solutions prior to the next centrifugation. The preferred processes according to this invention also advantageously include similar washing, centrifugation, and supernatant removal cycles using washing solutions similar to those indicated above, after inactivation of the enzyme digested starch intermediate.

The concentration and purification procedures according to this invention further advantageously include steps for separating purified portions rich in the resistant starch from relatively less pure portions. The separation is preferably performed before and after inactivation of the enzyme. It has been found desirable in some cases to use a cold water wash prior to the separation since the centrifugation mass tends to form two layers of differing colors. The enzyme-rich fractions have been found by the inventors to be concentrated by centrifugation as a relatively denser phase having darker color which migrates to the outer portions of the centrifugation chamber or bottom of centrifuge tubes. The desired product is of lighter density and lighter color and can be separated from the less desirable heavy fraction which contains residual inactivated enzyme and by-products of the enzyme digestion process, as the case may be. Mechanical separation can advantageously

be performed after suitable washing procedures as explained above to remove soluble contaminants without substantial loss of the relatively insoluble resistant starch being sought.

To perform the separation, the centrifugation mass is first alleviated of the supernatant from the previous washing step, which is typically discarded. The resulting centrifugation mass is then divided to separate the color graded portions. Alternatively, it may be possible to grade on the basis of relative position in the centrifuge chamber or other appropriate techniques which may be appropriately calibrated for the particular stage and purification processing being employed. Graded fractions which have been separated as undesirable can be recombined and reprocessed to extract the desirable components contained therein, such as by rewashing and centrifugation to discriminate a lighter fraction from a heavy fraction. This reprocessed resistant starch-containing material is then separated with the lighter fraction reserved and the heavy fraction eliminated. The processes of this invention using such recombination and refractionation can be repeated as necessary to increase the obtainable yield from the production processes.

In the above separation techniques, the enzyme contaminated fraction was present at the bottom of the centrifugation tube. It is also possible to obtain a thin enzyme contaminated fraction at the top of the centrifugation tube, particularly after the enzyme has been heat inactivated. After autoclaving for inactivation, the samples were allowed to cool and were centrifuged. In this case a thin layer of denatured enzyme was present on top of the starch in the centrifugation tube. However, no separation of the bulk of the starch into two layers took place.

After centrifugation of the hot inactivated mixture, it is preferred to wash only the surface of the previously centrifuged dense starch mass with washing solutions after removing the supernatant. In particular, it has been discovered that such a top washing approach is suitable to remove a thin layer of darkened denatured enzyme and advantageous when using an approximately pure or 95% ethanol washing step significant in minimizing the number of washings needed to produce a resistant starch product of desired purity and white color. Such top washings with water, water and ethanol, or ethanol can be utilized once in the purification, or numerous times as the case may be appropriate. Such top washing procedures are particularly appropriate when the washing procedure is utilized after inactivation, i.e. denaturation of the enzyme and prior washing cycles with cold water used to create a thin layer of denatured enzyme and to partially remove the hydrolyzed fractions of the enzyme digested starch intermediate. Subsequent washing with hot water is performed to remove more effectively hydrolyzed starch fractions.

In some uses of the purified resistant starch products made by this invention it will be suitable to use the product in a wet form still containing substantial amounts of water, such as from the purification processing described above. However, in other uses it is desirable to have a resistant starch product which is relatively dry, such as in a dry powdered form. Such dry forms of the novel purified resistant starch products advantageously have improved storage capabilities with great stability. The drying of the wet purified resistant starch product is advantageously accomplished by first extracting excess water through centrifugation. The resulting water extracted product is then advantageously dried by exposing such product to evacuated pressures below atmospheric pressure. Vacuum pressures are preferably below 100 millitorr. The temperatures during drying are preferably in the approximate range 10°-60°C. Drying times depend upon the conditions (batch size, vacuum dryer capacity, etc.). The residual moisture content of the dried product is preferably in the range 2-15%, more preferably 2-10%. Other drying techniques may alternatively be usable, but caution must be exercised concerning the potential derogatory effects of the type of drying used. For example, highly elevated temperatures often used in drying cereal products can lead to darkening and derogation of the quality of the desired resistant starch product produced by the novel processes of this invention.

The dried purified resistant starch products according to this invention can be stored at ambient temperatures preferably at temperatures in the range of less than 50°C, more preferably less than 30°C. The novel wet purified resistant starch products are preferably stored at temperatures in the range approximately less than 4°C, if necessary with preservatives to prevent microbial attack. Freezing of the wet starch product without preservatives may be appropriate but more expensive.

The purified resistant starch products made in accordance with this invention are typically white to slightly yellow in color. They are bland or flavorless which is important for use as an additive to other food products where introduction of a substantial flavor component may be undesirable, even where the additive has an agreeable taste. The resistant starch products produced by this invention are over 50% resistant starch as determined by analytical testing as described below in Example 1 and Example 3. When using the more preferred bacterial alpha-amylase, the purified resistant starch products of the invention ranged from 65% to over 80% resistant starch as determined by the same assay technique. When the resistant starch is made using preferred process parameters, RS levels of greater than 70% are regularly achieved. The remainder of the production product, not resistant starch is primarily unconverted starch.

The novel resistant starches produced by the inventive processes are distinctive in that a product is produced with high resistant starch, high purity, and excellent shelf life, even at ambient temperatures. Thus preferred products according to this invention have properties exceeding any prior art resistant starch preparation. Fig. 1 shows a scanning electron micrograph of raw amylomaize VII starch granules. Fig. 2 shows the same type of starch after being autoclaved and cooled for one (1) cycle. Fig. 4 shows the same type of starch having passed through four (4) autoclaving and cooling cycles, and is indicative of a somewhat more compact structural form of the starch. Figs. 3 and 5 show thermograms from differential scanning calorimetry reflecting the endothermic melting enthalpies of the treated starches presented in Figs. 2 and 4, respectively. Fig. 6 shows a scanning electron micrograph of a resistant starch product produced according to this invention from the crude heat-moisture treated starch illustrated in Fig. 4. There are significant apparent structural differences versus the crude starch preparation illustrated in Fig. 2. The porous structure, characteristic of the crude starches, disappeared and was most likely removed by the enzymatic treatment. Fig. 8 shows an electron micrograph of a separated residue removed during the purification of the preferred resistant starch product. Differences among crude starch and corresponding purified RS products are further emphasized by the differential scanning calorimetric thermograph presented in Figs. 7 and 9 versus similar information presented in Figs. 3 and 5. More complete description of such differences will now be made in connection with Figs. 10A and 10B.

Fig. 10A shows four differential scanning calorimeter thermograms for samples of amylomaize VII starch which was heat treated in an autoclave and then cooled, in case D repeatedly to 4 times. All treatments were done with starch:water ratios of 1:3.5. The line labelled A is associated with starch processed 1 cycle at 148°C. The line labelled B is for starch processed 1 cycle at 121°C. The line labelled C is for starch processed 1 cycle at 134°C. The line labelled D is for starch processed 4 cycles at 134°C.

Fig. 10B shows four related differential scanning calorimeter thermograms for purified RS products prepared according to the invention from the same starches as indicated above. The dramatic differences between the related lines A-D in Fig. 10B compared to the similarly labelled lines A-D in Fig. 10A, clearly provide additional evidence of the substantial differences between the crude heat processed retrograded starch products of the prior art and the novel purified resistant starch products of this invention. Fig. 10B indicates that for all starch heat treatment procedures used there were greatly higher amounts of heat energy required to melt the purified product compared to the crude starch preparation. This is indicative of the much greater heat stability of the purified products as

compared to the crude product. Table 2 further indicates quantitative information provided by the graphs shown in Figs. 10A and 10B. The temperature Ti indicates the initial transition temperature: the start of the endothermic melting peak. Temperature Tp indicates the temperature at maximum. Temperature Tc indicates the temperature associated with completion of melting. The enthalpy change is representative of the total energy input required to melt amylose crystallites, which represent enzyme resistant starch. Information on RS content and thermal characteristics is shown for both the crude, heat and moisture treated starch, and purified resistant starch products.

TABLE 2

Thermal Characteristics and RS Contents of Heat-Treated Amylomaize Starch and Purified Resistant Starch Products

Number of Autoclaving/ Cooling Cycles	Autoclaving Temperature (°C)	Resistant Starch Content (%)	Transition Temperatures (°C) Ti	Tp	Tc	Melting Enthalpy (J/g)
		Amylomaize VII Starch				
1	148	20.0	131.2	154.2	161.3	1.9
1	121	24.6	125.8	149.1	160.1	2.8
1	134	21.3	131.9	149.3	163.4	2.7
4	134	31.8	132.1	152.9	162.2	8.8
		Resistant Starch Products				
1	148	57.4	125.9	153.6	163.6	12.2
1	121	72.7	120.7	150.6	163.3	19.5
1	134	71.0	124.3	152.7	163.4	19.3
4	134	80.7	124.5	154.5	165.3	31.3

The increased energy which must be used to melt the purified product is indicative of the storage life at room temperatures and the likelihood that derogatory flavor changes might occur with storage time. Thus the novel products can be used in relatively high temperature food processing applications wherein the food is exposed to short-term heat, and act as a temperature moderator; for example, extrusion cooking at not too high temperatures.

Additional distinctions between the purified resistant starch preparations and unpurified heat treated and gelatinized starch are presented below.

EXAMPLE 1

The amount of RS in the product depends, among others, on the amount of RS in the crude heat-moisture treated starch preparation. Therefore, information was sought on the variations in amounts of RS in the crude starch preparation due to variation in the process parameters of starch:water ratio, gelatinization temperature, and number of cycles of autoclaving, and cooling. The selected starch was commercial grade amylomaize VII. The heating and cooling procedures were performed once in some tests and four times in other tests, at heating temperatures of 121°C, 134°C, and 148°C in an autoclave. The heat treated starch-water samples were allowed to cool to room temperature overnight to allow retrogradation to take place. The results in terms of the percent resistant starch content of the retrograded crude starch gels are indicated in Fig. 11. The resistant starch content was determined using a standard enzymatic assay typically used to determine content of dietary fiber in foods, according to standard procedures promulgated by the Association of Official Analytical Chemists (AOAC), Washington, D.C., under AOAC Method 43.A14. Resistant Starch contents increased from temperature 121°C to 134°C, and decreased again at 148°C. Starch:water ratios of 1:3.5 and 1:10 were used for each temperature and for 1 and 4 processing cycles.

Fig. 11 shows that best results were obtained at temperature 134°C with four heating and cooling cycles and a starch:water ratio of 1:3.5. The starch ratio 1:3.5 outperformed 1:10 in all cases. Four cycles outperformed one cycle in all cases.

EXAMPLE 2

Crude starch gel prepared as indicated in Example 1 with a single heating and cooling cycle was comminuted in a blending device and Takalite brand bacterial alpha-amylase was added to the resulting mixture in the amount of 5 milliliters of enzyme solution to 100 grams of heat processed and gelatinized water-starch gel in each sample. The enzyme was mixed into the sample to homogeneity and allowed to digest for approximately 60 minutes. The enzyme in the digesting samples was heat inactivated by placing the samples in an autoclave and heating to 134°C for the indicated time. The samples were tested for enzyme activity according to a colorimetric alpha-amylase assay. A (-) in Table 3 indicates that the sample showed no signs of active enzyme.

Table 3 also shows percent resistant starch fraction in the purified end product prepared according to the procedure set forth in Example 4.

TABLE 3

INACTIVATION OF BACTERIAL ALPHA-AMYLASE

Treatment Time (Minutes)	Purity of RS (%)	Enzyme Activity
5	72	-
10	72	-
15	71	-
20	71	-
30	69	-
60	66	-

5 milliliters Takalite per 100 grams starch

Inactivation temperature 134°C

EXAMPLE 3

Samples were prepared as indicated in Example 1 using 30 minutes heat treatments at 134°C and one cooling cycle to room temperature overnight. The resulting starch-water gels were enzymatically digested as indicated in Example 2 using digestion times of 60 minutes. The resulting digested starch-water mixtures were thereafter heat treated to inactivate the enzyme using treatment in an autoclave for 5 minutes at 134°C. The inactivated digested samples were purified using a preferred process for purification which was comprised of the following procedures.

The relatively hot, 80°-90°C, samples from the heat inactivation autoclaving step were centrifuged at approximately 1700 g for 10-15 minutes. The supernatant was removed by decanting from the sample vessels and the surface of the resulting sample starch preparation was washed with approximately 10-20 ml ethanol without stirring the bulk of dense starch mass. Ethanol was removed by decanting and the starch mass was mixed with 500 milliliters of cold water. The resulting mixture was centrifuged under the same conditions and the supernatant removed. The resulting samples were then treated again with ethanol by adding onto the resistant starch preparation samples approximately 10-15 milliliters of ethanol. After top washing and removing the ethanol, the samples were mixed with 500 ml cold water and centrifuged as before and the supernatant removed. Similar top washings with ethanol

were repeated for a total of 3 centrifugations. The supernatant was removed. Thereafter the samples were water washed by mixing the resulting samples with 500 milliliters cold water. The mixtures were then centrifuged as before and the supernatant removed. The cold water washing was repeated just as before again. Thereafter similarly conducted hot water washings (approximately 90°C) were made with intervening centrifugations and removal of supernatants.

The resulting samples were dried under vacuum for approximately 48 hours to obtain a residual moisture content of approximately 5%. The standard enzymatic assay indicated in Example 1 was conducted upon the samples and a resistant starch content in the purified product of 70.8% was achieved. The yield was calculated to be approximately 16.2% by comparing the weight of the purified resistant starch product to the 100 gram weight of the starch used to prepare the sample. The resulting product was white and had no detectable odor.

Testing was also conducted incorporating pancreatic or fungal alpha-amylase instead of bacterial alpha-amylase and using equivalent amounts and processing conditions required for these enzymes. The resulting RS products had substantially lower RS contents and subsequently higher amounts of still degradable starch structures were obtained for the two products (Table 4). Thus, the application of different enzymes provides different types of products showing most likely differing functional and physiological properties.

TABLE 4

APPLICATION OF DIFFERENT ENZYMES

	Recovery (%)	RS Content (%)	RS Yield (%)
Takalite or Termamyl (bacterial alpha-amylase)	19	71	16.2
Clarase (fungal alpha-amylase)	39	56	21.8
Pancreatin (porcine alpha-amylase)	48	38	18.2

Recovery: amount of RS product from 100 g amylomaize VII.

RS content: RS content of the product.

RS yield: Recovery x RS content.

EXAMPLE 4

Samples were prepared as indicated in Example 3 and purified using substantially the same purification procedure, the difference being the use of hot water in lieu of ethanol as top washing solution. The resistant starch content was 70.2% and the yield was 16.7%. The resulting product was slightly yellow and had a faintly detectable odor.

EXAMPLE 5

Samples were prepared as indicated in Example 4, the difference being that four cycles were used instead of one autoclaving/cooling cycle. The resistant starch content was 80.7%.

EXAMPLE 6

Samples were prepared as indicated in Example 3 and purified using a procedure as now indicated. The inactivated digested samples were centrifuged after cooling to room temperature overnight. The supernatant was removed and the remaining samples were each mixed with 500 milliliters of cold water and centrifuged. This mixing, centrifugation, and supernatant removal process was repeated 8 times. After each centrifugation step, the resulting starch mass showed 2 different colored layers, the lower with a darker color, the upper with a whiter color. The lighter upper portions from the samples were carefully

removed from the centrifugation container and were dried under vacuum as indicated above in Example 3. The darker layer was remixed with 500 ml water. After centrifugation, the 2 formed layers were separated by removing the desired white top layer. The standard assay indicated in Example 1 was used to obtain a measurement of 66.0% resistant starch in the produced product. The yield was 17.2%. The product was slightly yellow and had a faintly detectable odor after storage for 7 days.

EXAMPLE 7

Samples were prepared as indicated in Example 6. Prior to inactivation by heat as indicated in that Example, the samples were washed once with 200 ml 95% ethanol and three times with 500 ml cold water. Thorough mixing and centrifugation followed removal of supernatant. The samples were then autoclaved for 5 minutes at 134°C to inactivate the enzyme. Thereafter a series of 5 washings using cold water were performed using substantially the same washing, centrifugation and supernatant removal procedures as indicated in Example 6. The resulting centrifuged starch mass showed 2 layers which were divided as indicated in the previous Example and similar enzymatic assays were run. The resulting resistant starch content was 67.3%, and the yield was 16.8%. The resulting product was white and had no detectable odor.

EXAMPLE 8

Samples were prepared as indicated in Example 7. Prior to inactivation by heat as indicated in that Example, the samples were pre-inactivation washed using a washing solution. The washing solution was cold water. Ethanol washing was omitted. The pre-inactivation washings including mixing with wash solution, centrifugation, and supernatant removal were repeated 4 times. The samples were then autoclaved for 5 minutes at 134°C to inactivate the enzyme. Thereafter a series of 5 washings using hot water were performed using substantially the same washing, centrifugation, and supernatant removal procedures as indicated for the pre-inactivation washes. The resulting centrifuged starch mass showed no layering and the samples were not divided as indicated in the previous Example. Similar enzymatic assays were run. The resulting resistant starch content was 74.1%, and the yield was 16.5%. The resulting product was slightly yellowed and had some detectable odor.

EXAMPLE 9

Samples were prepared as indicated in Example 8. Prior to inactivation by heat as indicated in that Example, the samples were pre-inactivation washed using a washing solution. The washing solution was hot water. The pre-inactivation mixing with wash solution, centrifugation, and supernatant removal were repeated 4 times. The samples were then autoclaved for 5 minutes at 134°C to inactivate the enzyme. Thereafter a series of 5

washings using hot water were performed using substantially the same washing, centrifugation and supernatant removal procedures as indicated for the pre-inactivation washes. The resulting centrifuged starch mass showed no layering and the samples were not divided as indicated in the Example 7. Similar enzymatic assays were run. The resulting product had a resistant starch content of 74.6%, and the yield was 15.7%. The resulting product was slightly yellowed and had no detectable odor.

EXAMPLE 10

Samples were handled as indicated in the previous Example 9 except the pre-inactivation washing solution was 95% ethanol (once) and water at about 70-90°C (three times) with centrifugation and removal of supernatant. Thereafter, a series of 4 washings using hot water was performed. The resultant purified product had 76.1% resistant starch; the yield was 13.5%. The resistant starch was white and had no smell.

EXAMPLE 11

Samples were prepared from a number of different starch feedstock materials as indicated in Table 5. The starch materials were heat treated as indicated in Example 1 for 1 cycle. Assays were conducted on each sample type after the heat treatment and cooling according to the procedure of Example 1. The results of such assays are shown in Table 5. Resistant starch products were prepared according to the process indicated in Example 3. The resulting purified samples were assayed using the same assay and the concentration results are shown in Table 5. Table 5 also indicates the recovery or yield of resistant starch in the resulting purified samples. The superior results of the high amylose containing amylomaize starch are clearly evident.

TABLE 5

YIELDS OF RESISTANT STARCH
FROM ALTERNATIVE STARCH SOURCES

Starches	RS Content (%) Crude Heat-Moisture Treated Starch (1 Cycle)	RS Product	Recoveries of RS Products (%)
Amylomaize VII	21.3	70.8	16.2
Pea	10.5	66.0	7.8
Maize	7.0	63.1	3.0
Wheat	7.8	59.6	3.2
Potato	4.4	48.6	0.9

Some of the common sources for production of dietary-fiber (DF)-enriched or caloric-reduced foods include bran from wheat, barley, corn (maize), oat, soy, or pea processing; legume non-starchy polysaccharides; fruit fibers (apples, sugar beets); and powdered cellulose, gums, and related materials. Gums are used in too small amounts to have a physiological effect; in large amounts they create problems in food processing (agglomeration, formation of lumps, very rapid water absorption).

The above materials, except for cellulose and gums, have specific flavors, tastes, and colors which at high levels are, generally objectionable in food processing and limit their incorporation in foods.

Production of dietary fiber or calorie reduced material of desirable food technological and physiological characteristics has been attempted by a combination of the following:

a) blending of ingredients from various sources and varying widely in properties,

b) masking or eliminating undesirable properties (e.g. bleaching, removing objectionable flavors or odors; extensive processing).

Thus, for instance, wheat bran may be washed and treated, chemically and/or enzymatically, to remove phytic acid, pesticides, and heavy metals; boiled to inactivate heat labile components; and dewatered-dried to produce a stable product.

Still, the extensively processed product may be at best a compromise in terms of desirable properties; tailoring to impart the best combination of characteristics may be impossible to attain. Consequently, cellulose was used for many years because of the very high concentration of dietary fiber in a technologically acceptable food product. Both the image-perception of wood cellulose and its limited physiological function have resulted in discontinuation of its inclusion in most foods.

The novel resistant starch products have additional uses because of the greater purity and more stable nature. In addition to their extended shelf life as compared to the unpurified starches, they show less detrimental effects when used as additives in other food products, and thus are of greater applicability and utility as food additives than relatively unpurified resistant starch preparations.

In addition to their effect as dietary fiber or low caloric food additive, the novel purified resistant starches of this invention can at the same time be used as a hydrating agent to absorb water in various food products to adjust the processing of such products, such as in cooking, cooling, and other facets of food processing. The particular resistant starch product can be tailored to a particular use by adjusting the processing or blending with non-resistant starch products as needed. For example, the water absorbance of the purified resistant starches is high (4 g water/g product). Accordingly, the concentration of RS can be adjusted either during purification or by blending after purification to achieve a particular level of hydrating capacity. The novel products can also be used as heat absorbing additives as explained hereinabove.

The novel products according to this invention can be used in the preparation of diverse baked goods and other foods to act as a dietary fiber equivalent or similar material, or to act in any of the other indicated beneficial ways. Examples of foods which can potentially include the novel purified resistant starch products include bread, cookies, pasta, baked goods, dairy products, beverages, puddings, fillings, and others. Exemplary examples are given below. The products can also be used as a dietary additive to lower the caloric content of the foods to which they are added. The purified resistant starch products can be added in varying amounts, for example, 0-20% of the food product, more preferably 5-10% of the food product. As a dietary supplement, for example in obesity control, the novel products of this invention can be administered as a purified powder or tableted and taken as a pill.

The novel resistant starch products are relatively low in contaminants versus other sources of dietary fiber currently in widespread use which can contain various bacteria, fungi, heavy metals, insecticides, and other pesticides. The novel products also contain little or no

lipids or phytic acid. The novel products of this invention can also be used as a dietary supplement. The products can be administered in powdered, capsule, tablet or caplet form, preferably in dosages of 0-10 grams, more preferably 1-5 grams.

EXAMPLE 12

A purified resistant starch product of this invention having approximately 70% resistant starch content was used in the preparation of Japanese Udon noodles. The product was added as a component of the flour used to prepare the noodles in amounts sufficient to provide a concentration of the 70% resistant starch product equal to 4% of the total flour. Comparative samples were also made using wheat bran, soy bran, and a control using pure flour. The noodles were made in the typical manner and four characteristics of the noodles were evaluated, namely: color of the raw noodle; color of the cooked noodle; texture of the cooked noodle; and, yield. The resistant starch containing noodles scored higher than either the wheat bran or soy bran in combined score for the 4 characteristics. The resulting noodles were white, non-gritty, and acceptable substitutes of the normal noodles made without the purified resistant starch products or for noodles enriched with soy fiber or wheat bran.

EXAMPLE 13

Cookies were also prepared and baked using standard cookie flour supplemented to provide 3%, 5% and 7% concentrations of the 70% purified resistant starch product. Compared to similar levels of wheat bran or soy fiber, the incorporation of the novel additive reduced the cookie diameter and provided lighter colored cookies. Top grain cracks were reduced with increasing concentrations of resistant starch product. The resulting cookies were acceptable substitutes for the normal cookies made without the purified resistant starch products, or for wheat bran and soy fiber enriched cookies.

Industrial Applicability

The novel purified resistant starch products produced by the processes of this invention can be used as dietary supplements either in their purified form, mixed forms with other dietary materials, or as ingredients in novel food products. The purified resistant starch products can be used as additives in food products to substitute for conventional dietary fiber sources, produce foods with lower caloric content, serve as water binding ingredients, control water distribution, heat absorbing and other functions. The products are superior in sensory properties, stability, and storage capability versus unpurified resistant starch preparations. The concentrated resistant starch products can also be administered in their purified form as tablets, capsules, or caplets as a dietary supplement affecting gastrointestinal tract function.

CLAIMS

1. A method for producing a purified resistant starch product with high resistant starch content, comprising:

mixing a starch-containing material with water to form a water-starch mixture;

heating the water-starch mixture above 100°C;

cooling heated water-starch mixture to form a retrograded starch gel;

comminuting the retrograded starch gel;

mixing an amylolytic enzyme into the water-starch gel;

allowing the amylolytic enzyme to digest portions of the starch contained in the starch gel to produce a digested water-starch mixture;

heating the digested water-starch mixture to temperatures sufficient to inactivate the amylolytic enzyme and produce inactivated digested water-starch mixture;

concentrating resistant starch by removing digested starch fractions.

2. A method according to claim 1 and further comprising, purifying the resistant starch fraction from the inactivated digested water-starch mixture to produce a purified resistant starch product.

3. A method according to claim 1 and further comprising, removing water from the separated resistant starch fraction to produce a dry purified resistant starch.

4. A method according to claim 3 wherein said removing water is accomplished by treating resistant starch fraction using vacuum pressures.

5. A method according to claim 1 wherein the amylase is heat stable alpha-amylase.

6. A method according to claim 1 wherein the amylase is bacterial alpha-amylase.

7. A method according to claim 1 wherein the amylase is heat stable bacterial alpha-amylase.

8. A method according to claim 1 wherein said mixing a starch-containing material is with water and starch-containing material at ratios in the approximate range of 1:3.5 to 1:10, starch:water.

9. A method according to claim 1 wherein said heating the water-starch mixture is to at least 120°C.

10. A method according to claim 1 wherein said heating of the water-starch mixture is to approximately 134°C.

11. A method according to claim 1 wherein said comminuting is by a wet milling process.

12. A method according to claim 1 wherein enzymic conversion of a degradable starch structure is performed for 30 to 60 minutes at approximately 100°C with mixing of a starch-water-enzyme mixture.

13. A method according to claim 1 wherein said heating the digested water-starch mixture is carried out at temperatures above 100°C in order to inactivate the amylolytic enzyme.

14. A method according to claim 1 wherein said concentrating is by centrifugation and water washing of the inactivated digested water-starch gel to remove soluble starch fractions and leave concentrated resistant starch fractions.

15. A method according to claim 1 wherein the concentrated resistant starch is purified by a combination of washing with hot water, surface washing with ethanol, and centrifugation.

16. A purified resistant starch product comprising at least 50% resistant starch.

17. A purified resistant starch product according to claim 16 and further defined as having resistive starch content of at least 60%.

18. A purified resistant starch product according to claim 16 and further defined as having resistive starch content of at least 70%.

19. A purified resistant starch product according to claim 16 and further defined as having resistive starch content of at least 70% and further defined by processing including repeated autoclaving/cooling cycles.

20. A purified resistant starch product according to claim 16 and further defined as having resistive starch content consisting of at least 70%, and further characterized by endothermic enthalpy change in excess of twice the associated endothermic melting enthalpy of a crude retrograded starch gel.

21. A purified resistant starch product according to claim 16 and further defined to be derived from amylomaize starch.

22. A purified resistant starch product produced according to the following process:

mixing a starch-containing material with water to form a water-starch mixture;

heating the water-starch mixture above 100°C;

cooling the heated water-starch mixture to form a retrograded starch gel;

comminuting the water-starch gel;

mixing amylolytic enzyme into the water-starch gel;

allowing the amylolytic enzyme to digest portions of the starch contained in the water-starch gel to produce digested water-starch mixture;

heating the digested water-starch mixture to temperatures sufficient to inactivate the amylolytic enzyme and produce inactivated digested water-starch mixture;

concentrating the resistant starch fraction by removing digested starch fractions.

23. A purified resistant starch product according to claim 22 wherein said process further comprises purifying the resistant starch fraction from inactivated digested water-starch mixture to produce a purified resistant starch product.

24. A purified resistant starch product according to claim 22 wherein said process further comprises:

removing water from the separated resistant starch fraction to produce a dry purified resistant starch.

25. A purified resistant starch product according to claim 22 wherein said amylase is heat stable bacterial alpha-amylase.

26. A purified resistant starch produce according to claim 22 wherein said mixing is with water and starch-containing material at ratios in the approximate range of 1:3.5 to 1:10 (starch:water).

27. A purified resistant starch product according to claim 22 wherein said heating of water-starch mixture is to at least 120°C.

28. A purified resistant starch product according to claim 22 wherein said heating the digested water-starch mixture is carried out at temperatures above 120°C.

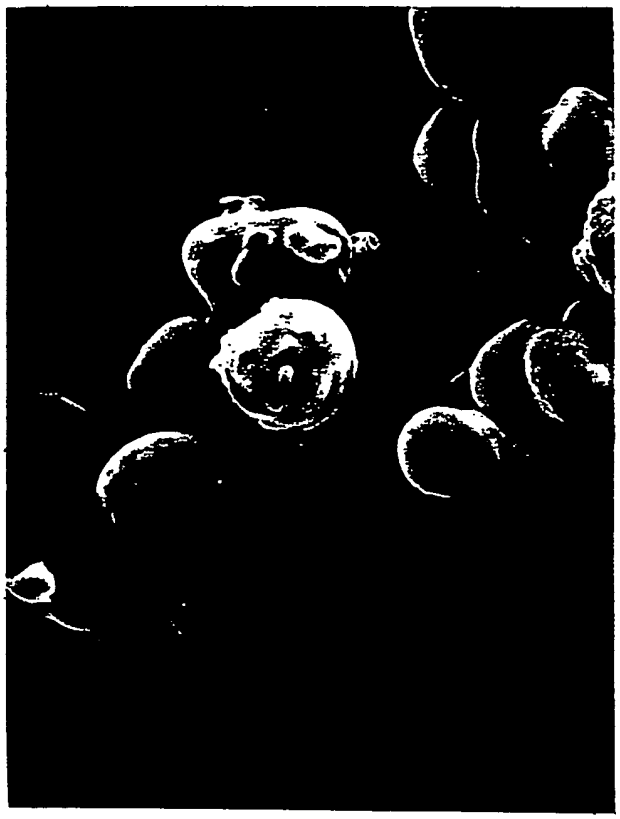

Fig 1

Fig 2

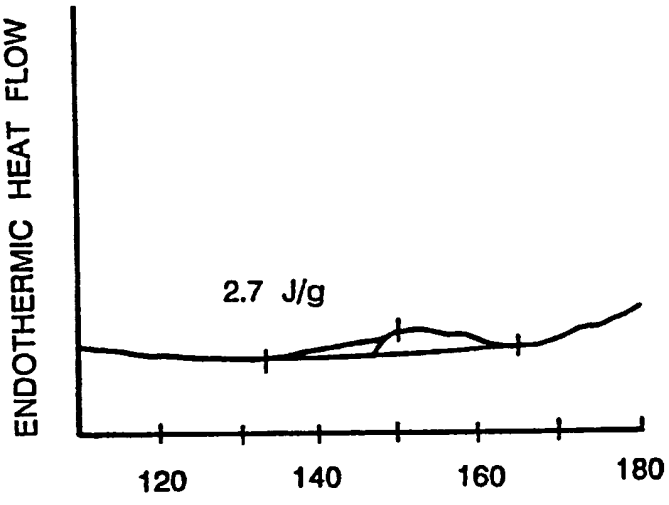

Fig 3

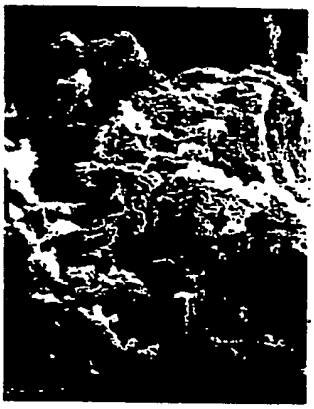

Fig 4

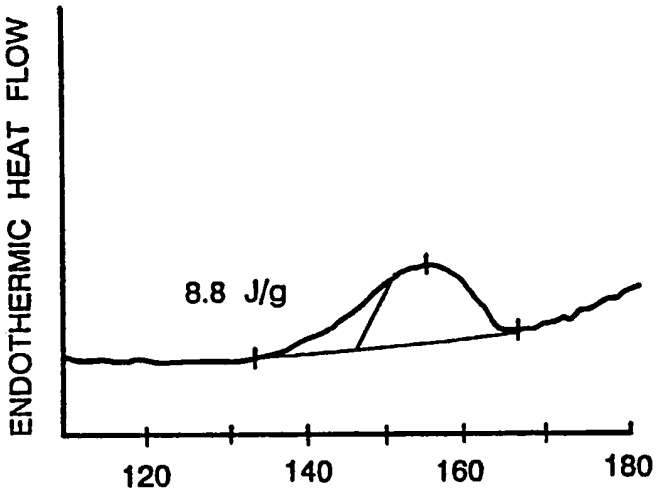

Fig 5

Fig 6

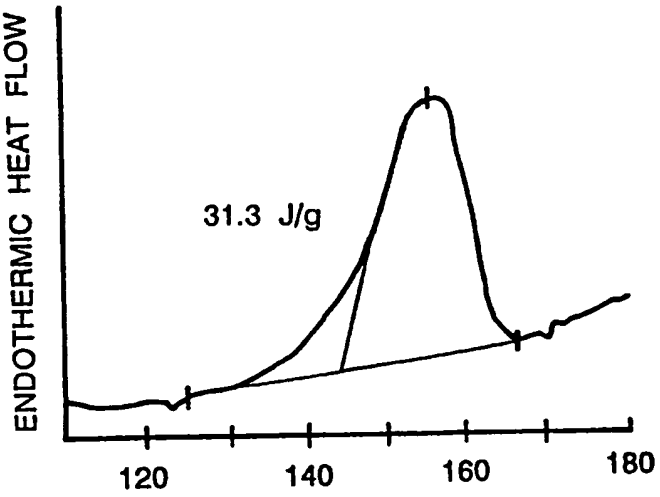

Fig 7

Fig 8B

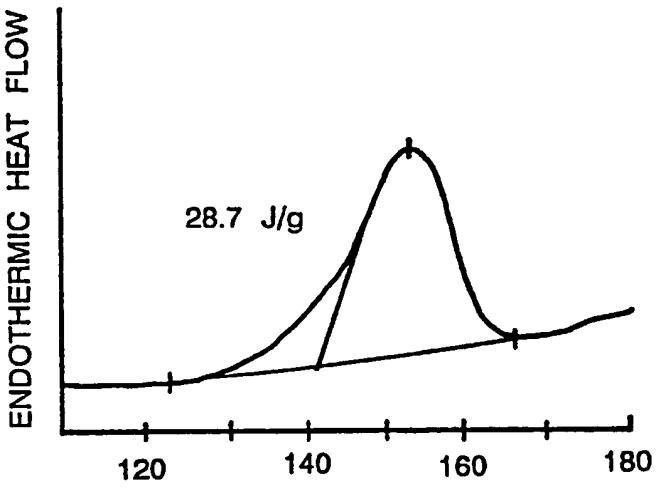

Fig 9

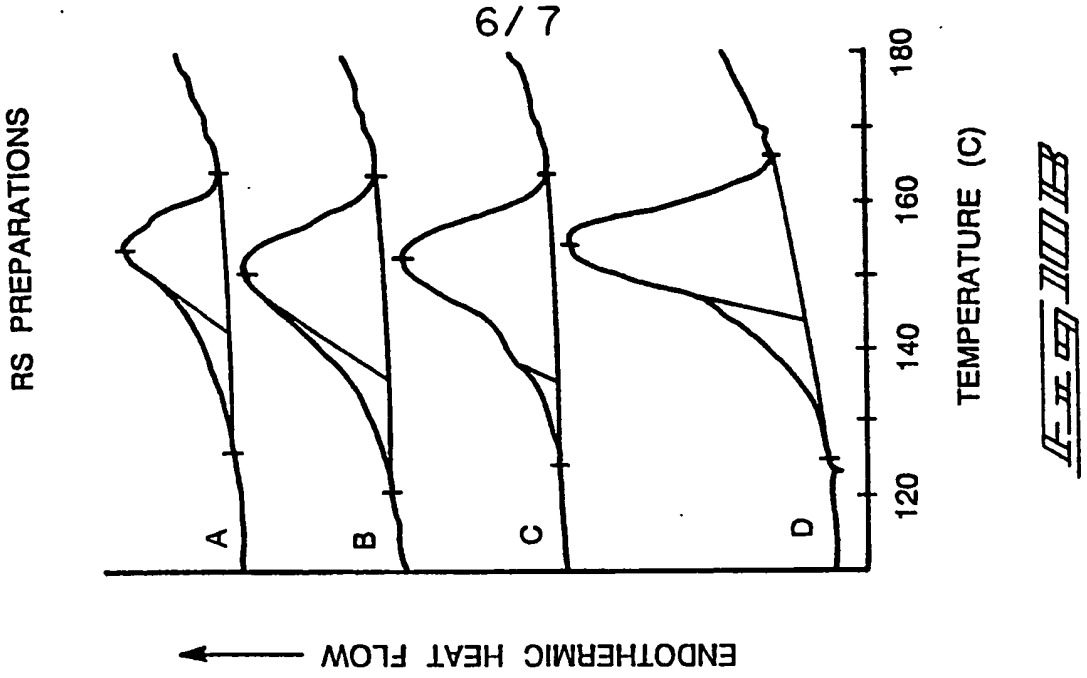
RS PREPARATIONS
A
B
C
D
120
140
160
180
TEMPERATURE (C)
ENDOTHERMIC HEAT FLOW

FIG 3B

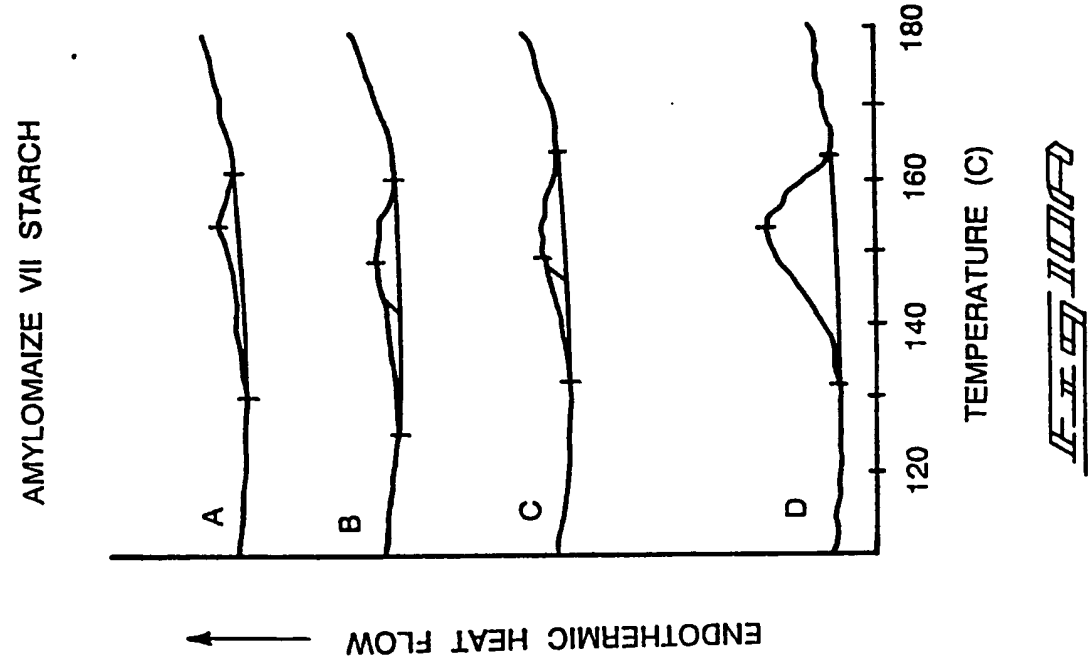
AMYLOMAIZE VII STARCH
A
B
C
D
120
140
160
180
TEMPERATURE (C)
ENDOTHERMIC HEAT FLOW

FIG 3A

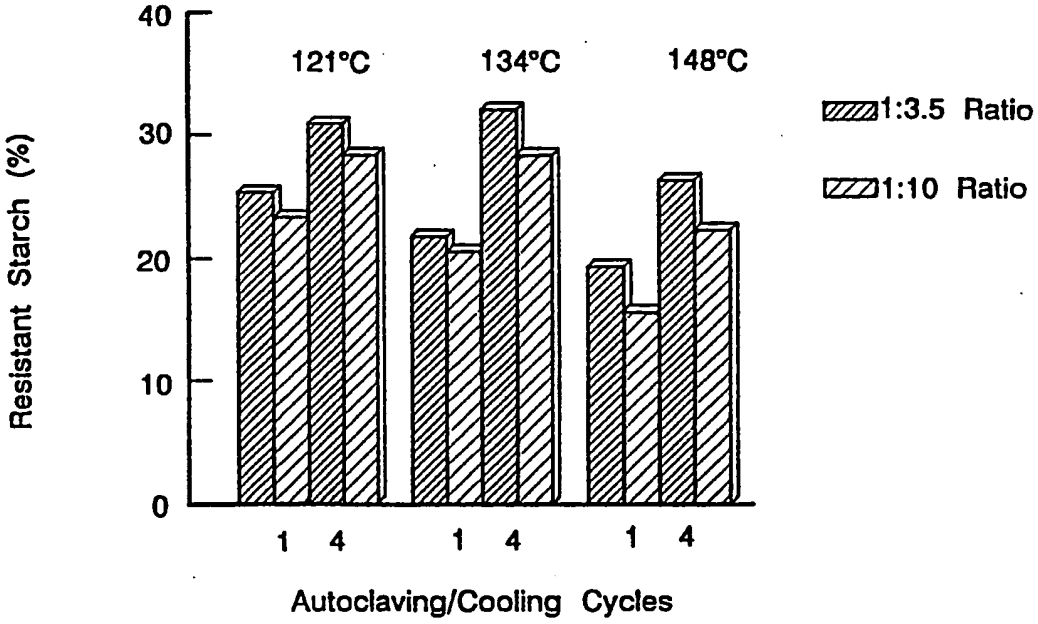
40
30
20
10
0
Resistant Starch (%)
121°C
134°C
148°C
1:3.5 Ratio
1:10 Ratio
1 4
1 4
1 4
Autoclaving/Cooling Cycles

Fig 11

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03205

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12P 19/14; C07H 3/00
US Cl.: 435/99

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	435/99

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Computer Searched CAS (1967-1990), Biosis (1969-1990), APS (1975-1990).
Terms: resist? starch; amylase or amylolytic

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y/X	Starch/Starke, Volume 41, Number 4, April 1989, Siljestrom et al, "Characterization of Resistant Starch from Autoclaved wheat Starch", pages 147-151, see entire document	1-15/16-28
Y	Starch/Starke, Volume 32, Number 5, issued May 1980 Ikawa et al, "Changes in Some Preperties of Starch Granules of Maize Having Amylos-extender gene by Amylose Attach", pages 145-149, see entire document	1-28
Y	US, A, 3,853,706 (ARMBRUSTER), 10 December 1974 see column 2, lines 38-59.	1-15, 22-28

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
24 AUGUST 1990	26 SEP 1990
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	CAROLYN S. ELMORE